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More with less: Advances in flow and paper-based monitoring of nutrients in aquatic systems*

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Abstract: This paper highlights the importance of the collection of data that is of suitable quality and of appropriate frequency and/or spatial intensity for the monitoring of aquatic systems. The advantages of automated techniques, such as flow analysis, in monitoring are emphasized, as is the selection of parameters that address the objectives of monitoring. The potential of nascent microfluidic and paper-based analytical techniques as tools for water quality monitoring is examined.

Keywords: analytical chemistry; aquatic systems; flow analysis; nitrogen; nutrients; paper-based devices; phosphorus; water.

INTRODUCTION

Water quality is monitored for a variety of reasons; viz., to determine the suitability for human and livestock consumption, for regulatory purposes where waste is discharged under a license agreement, or for assessment of ecosystem condition. Implicit in the monitoring process is the need to couple data collection with data evaluation and the formulation of appropriate management responses to address particular problems or to improve ecosystem status. However, if the data collected are spatially or temporally unrepresentative, or are flawed because of the use of inadequate sampling, transport and storage protocols, or inappropriate analytical methods, then this measurement-assessment-management nexus fails [1].

For example, Fig. 1a shows how the use of a high-throughput, flow-based analytical system used for underway measurement of filterable (or dissolved) reactive phosphate in a coastal lagoon system provides a superior representation of the nutrient status compared with data collected using conventional hand sampling and laboratory-based analysis (Fig. 1b) [2]. High-resolution measurements of this type are invaluable in tracing the source and dispersal of nutrients, but can also provide valuable insights into biogeochemical processes that operate within an aquatic system, e.g., uptake or release by sediments and particulates, bio-uptake, etc.

Strategic monitoring, i.e., that which is designed to capture temporal events or spatial variations of concentration, is highly desirable for effective water quality assessment and management. It is also important that the parameter chosen for measurement is appropriate to the monitoring objective. Total P in wastewater treatment effluent is a useful general water quality management tool but may not be the best indicator of algal growth potential, whereas measurement of dissolved reactive P will arguably provide a better measure of readily bioavailable P and hence potential primary production.

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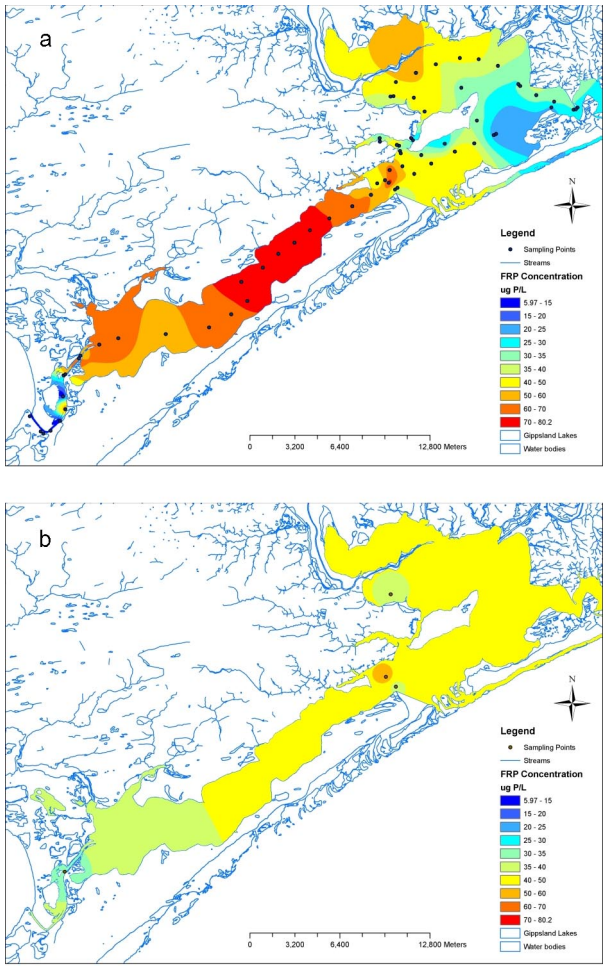

Fig. 1 Comparison of GIS maps for filterable reactive phosphate in surface waters of the Gippsland Lakes, SE Australia (a) based on 987 concentration values collected in <10 h using a flow analysis system deployed underway from a sampling vessel, and (b) based on 4 concentration values obtained from the same cruise using the routine sampling program of the local environmental agency. Reprinted from ref. [2].

Environmental chemists are often faced with the dilemma of performing environmental analyses using methods that have poor green chemical credentials, e.g., because the assays involve the use of toxic reagents, or the reagent and waste volumes used and produced are large. In this respect, flow analysis techniques are advantageous, and paper-based analytical systems offer even greater reagent savings. They have the added advantage that their portability also makes them very suitable for on-site analysis.

This paper illustrates how flow- and paper-based analytical techniques can be used for the assessment of water quality in a variety of water types to give *more* information with *less* cost, labor, and reagent consumption.

FLOW-BASED ANALYTICAL TECHNIQUES

Flow-based analytical techniques are used widely in nutrient analysis of environmental samples because they offer rapid sample throughput, with higher precision than can be achieved manually. Segmented

continuous flow techniques are still widely used by marine chemists, not necessarily because they provide the fastest or most precise measurements, but because they have become the unofficial benchmark techniques amongst the oceanographic community [3,4]. Arguably, flow injection or sequential injection techniques should enable nutrient determinations to be conducted with greater throughput by allowing better underway measurements or more economical reagent use (i.e., greener chemical practice) than is the case with segmented continuous flow analysis (SCFA). However, there has been an understandable reluctance on the part of some marine chemists to embrace these newer techniques in the absence of developed protocols and standardization, which even now are still being refined for SCFA methods [4]. In other areas of environmental analysis (e.g., waste and potable waters), there is perhaps wider acceptance of a variety of instrumentation and methods, but greater emphasis on quality assurance.

A recently reported flow-injection method for nitrate determination in estuarine and coastal waters provides a good example of an approach that embodies green chemical advantages and enables measurement of low concentrations with high spatial resolution. The conventional flow method for the determination of nitrate entails pre-reduction of nitrate to nitrite using a copperized Cd column, followed by reaction of the nitrite thus formed with sulfanilamide and a coupling agent (typically, *N*-(1-naphthyl)-ethylenediamine dihydrochloride) to produce a pink-colored azo dye that is detected by spectrophotometry. This forms the basis of many standard methods for the determination of nitrate in a variety of water types [5,6], despite a number of recognized flaws in the method (e.g., initial over-reduction, and subsequent under-reduction as columns age) [7,8], and the environmental and occupational hazards associated with the preparation and use of toxic Cd reduction columns [9]. Because of these acknowledged problems, there is now a trend in laboratory-based analysis toward the use of nitrate reductase, which despite its somewhat slower reduction kinetics [10,11] is much more environmentally benign than reduction with Cd.

In the flow analysis method proposed by Ellis et al. [12], a Zn column is used for complete pre-reduction of nitrate to nitrite, thus avoiding the use of Cd. The flow manifold is shown in Fig. 2, and was configured in a portable flow analysis system. This system was deployed aboard the vessel *Pelican1* and used during a cruise in Port Phillip and Western Port bays and Bass Strait (Australia) in January 2010 when more than 3200 individual time- and position-referenced determinations of nitrate were made at a rate of 40 h^{-1}.The resulting concentration-time data (Fig. 3) clearly illustrate the advantages of this approach, which enabled the observation of spatial concentration abnormalities that were due to point-source inputs from a series of waste outfalls and nutrient-enriched rivers and creeks. In a similar

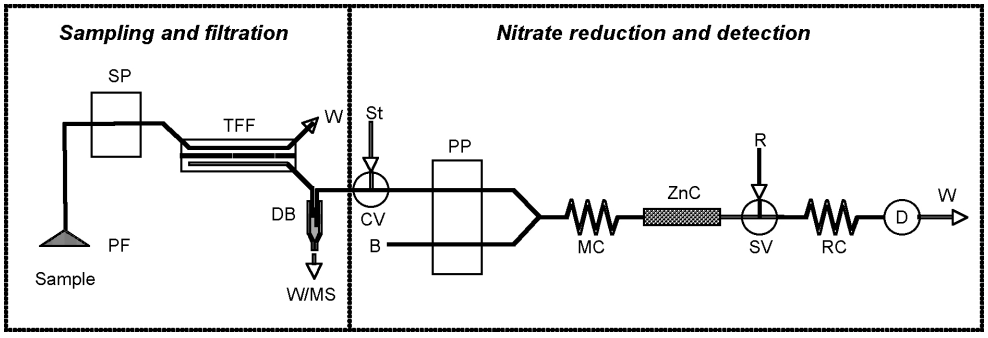

Fig. 2 Flow analysis manifold used for the determination of nitrate in coastal marine and estuarine waters. PF, prefilter; SP, sampling pump; TFF, tangential flow filter; W, waste line; DB, debubbler; W/MS, waste/manual sampling line; St, standard; CV, calibration valve; PP, peristaltic pump; B, buffer line; MC, mixing coil; ZnC, zinc column; R, Griess reagent; SV, solenoid valve; RC, reaction coil; D, detector. Reprinted with permission from ref. [12]. Copyright © (2011), Elsevier B.V.

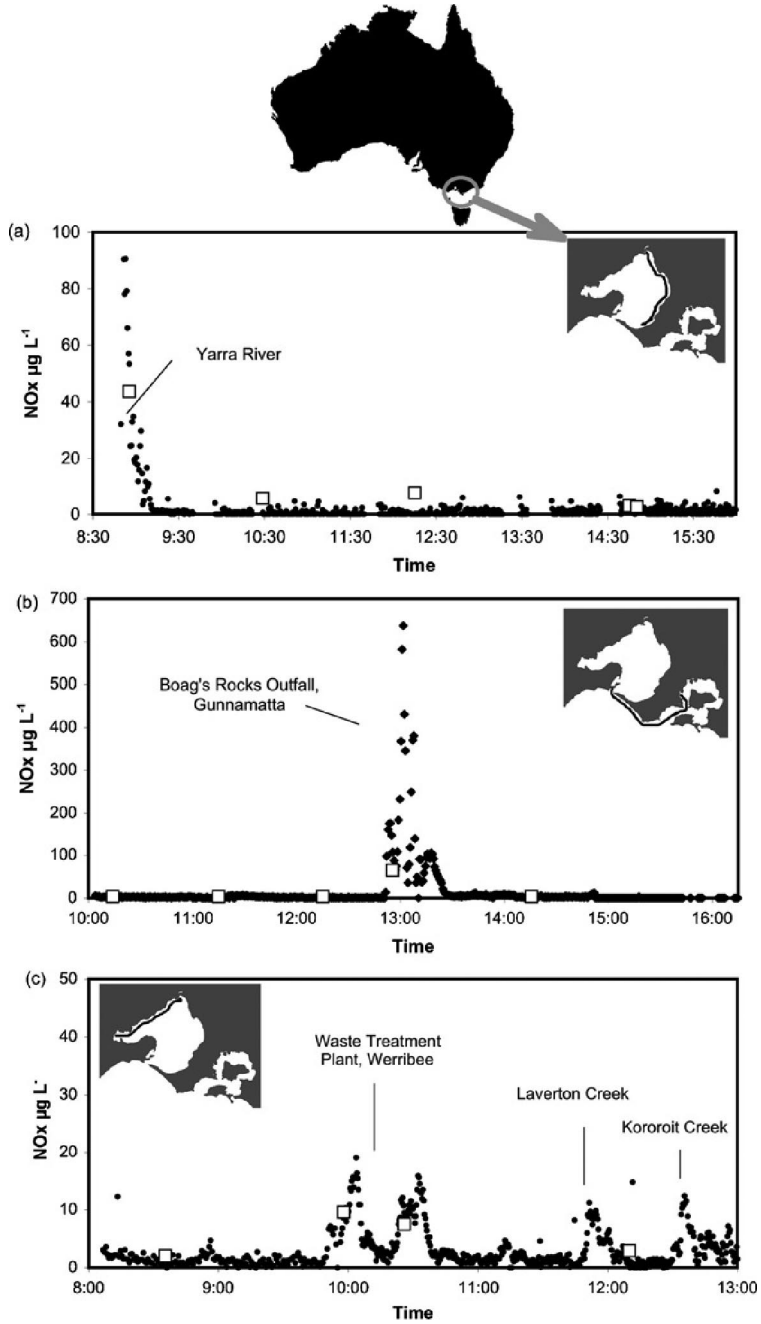

Fig. 3 Data from underway nitrate measurements in Port Phillip and Western Port bays and Bass Strait on (a) 11 January 2010, (b) 12 January 2010, and (c) 23 January 2010. High concentrations are observed in the Yarra River estuary (a), the outfall from the South Eastern Treatment plant (b), and the Western Treatment Plant outfall, and Laverton and Kororoit Creeks (c). ● points are for data measured in the field using the flow analysis system, while □ are for comparative results obtained from manual sample collection and subsequent and laboratory analysis. Reprinted with permission from ref. [12]. Copyright © (2011), Elsevier B.V.

exercise on the Derwent River estuary in Tasmania, Australia, 1400 discrete measurements of nitrate were performed at sea within 48 h. It was estimated that this number of determinations represented 46 working days if the same samples had been collected manually and determined by conventional oceanographic laboratory techniques.

Similar high-throughput, underway monitoring approaches using flow-based analysis have been demonstrated for the determination of dissolved reactive P [2,13–19], total P [20,21], and CO_2-related parameters such as total alkalinity [22–24], pCO_2 [25,26], and pH [27,28].

SPECIATION OF NUTRIENTS

An understanding of nutrient speciation in both water and sediment is important if the influence of spatial or seasonal changes in nutrient concentrations is to be related to eutrophication and the formation of harmful algal blooms (HABs). There is a range of reported flow-based techniques for nutrient speciation of sediments and waters. These include selective extraction of sediments in batch mode (e.g., sedimentary extraction (SEDEX) or similar techniques for the extraction of P [29]) followed by determination of the extracted analyte with automated flow analysis, and extraction of packed sediment/soil microreactors followed by analyte detection, all performed on-line using flow analysis [30]. For P, these techniques provide an indication of the size of the reactive P-pool that can be released from sediments and soils under differing environmental conditions such as reduction/oxidation, complexation, acid leaching, ion exchange, etc.

For the aqueous phase, speciation based on size discrimination or enzymatic reactivity provides similar information. For example, Turner et al. [31] and Monbet et al. [32,33] used off-line enzymatic hydrolysis of dissolved organic P with enzymes such as 3-phytase, alkaline phosphatase, and phosphodiesterase with flow-injection analysis of the phosphate produced to characterize samples according to their organic P enzyme substrates (Fig. 4). Others have used either single or multiple enzymes immo-

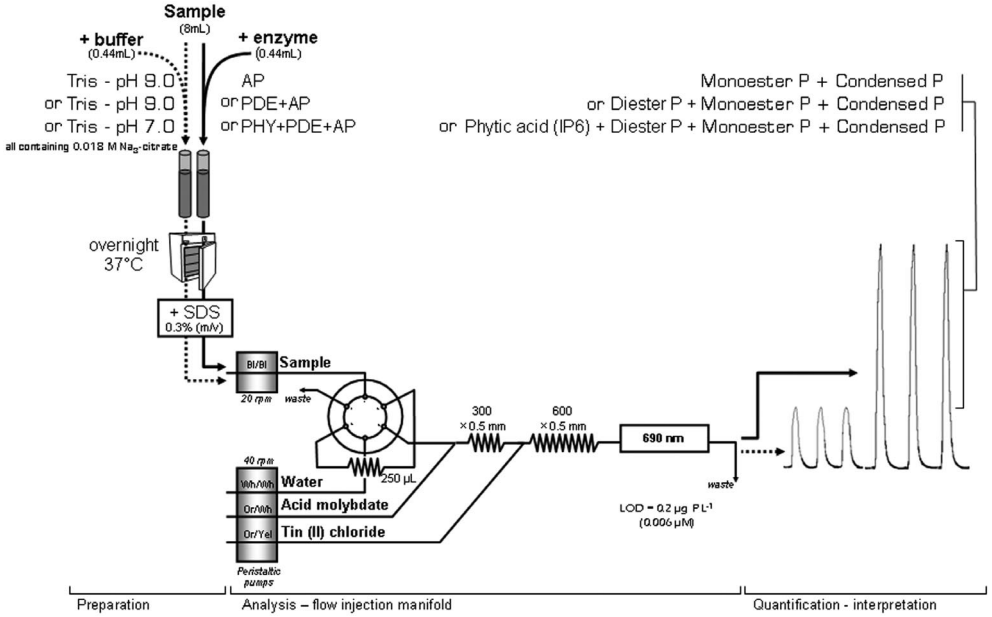

Fig. 4 Schematic summary of the procedure for enzymatic characterization of dissolved organic P in natural waters. The left side shows the enzyme incubation of the sample prior to determination by flow analysis, and quantification of the various components of enzyme-hydrolyzable P (EHP). Reprinted with permission from ref. [32]. Copyright © (2007) American Chemical Society.

bilized in packed-bed reactors in flow analysis systems to determine phospho-monoesters [34,35], phytate [36,37], and phospholipids [38] in waters and wastewaters. Arguably, the use of enzymes, either free or immobilized, in concert with flow analysis provides a more selective means of obtaining environmentally relevant information than empirically defined extraction methods, and in the examples cited can be used as an indication of the likely reactivity or bioavailability of organic P species. Similar approaches can be applied to the determination of dissolved organic N species [39].

The high repeatability and sensitivity of flow analysis techniques is advantageous when used in conjunction with enzymatic (or other) speciation schemes for dissolved nutrients because it enables resolution of different enzyme substrates that are often present in only small concentrations. This is illustrated in Fig. 5, which shows the relative abundance of organic and inorganic P present in waters of different salinities, as well as the various enzyme-hydrolyzable components of dissolved organic P.

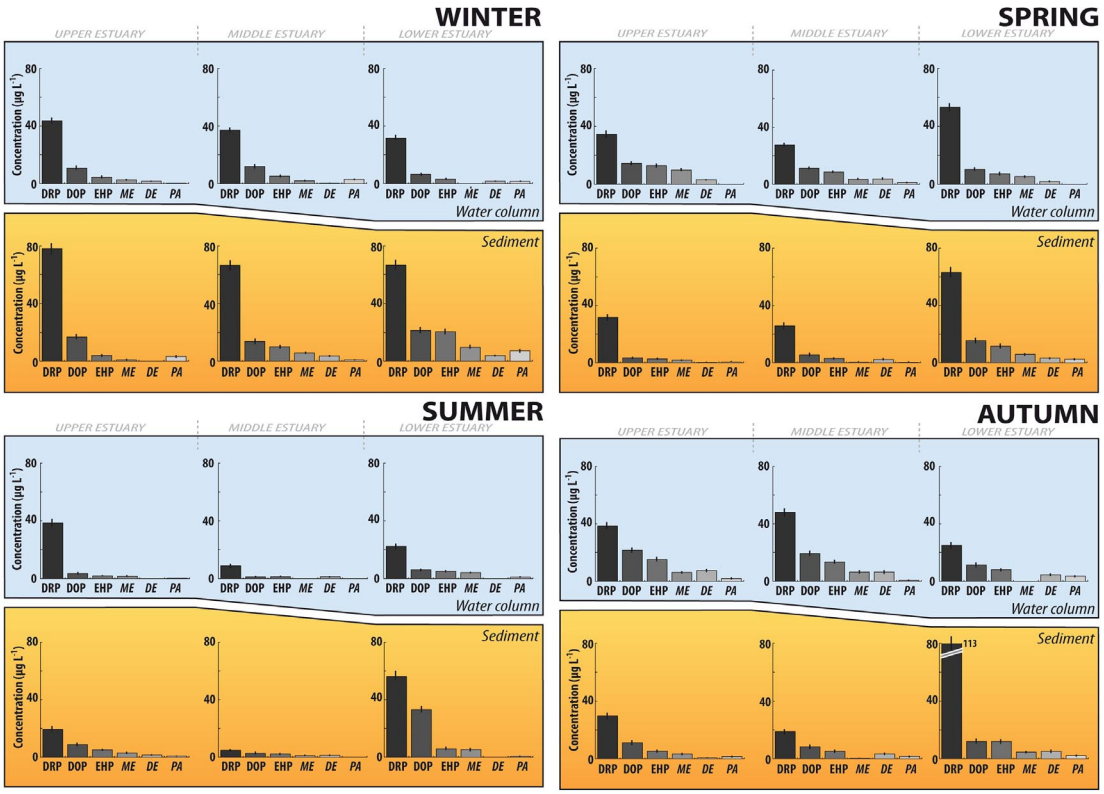

Fig. 5 Seasonal and spatial variation in P fractions in the water column and the sediment porewater at three locations in the Tamar river estuary, i.e., Sta. 2 = upper estuary; Sta. 4 = middle estuary and high turbidity zone; Sta. 8 = lower estuary. DRP = dissolved reactive P; DOP = dissolved organic P, EHP = enzymatically hydrolyzable P; ME = labile monoester phosphate; DE = diester phosphate; PA = phytic acid. Reprinted with permission from ref. [33]. Copyright © (2009) Elsevier B.V.

MICROFLUIDIC AND PAPER-BASED DEVICES FOR NUTRIENT MEASUREMENT

While flow analysis techniques have been shown to be suitable for fast, reagent-efficient determinations of water quality parameters in the laboratory, and to a lesser extent in the field, the perceived need for cheap, disposable analytical devices that use micro- or nano-litre volumes of reagents and samples with integrated detection has stimulated research in the area of miniaturization. The most common

approaches, lab-on-chip and μTAS (micro-total analysis system), usually involve the use of a chip or block of glass or polymer containing μm-size flow channels to minimize sample and reagent usage. Optical detection in these devices usually requires a microscope for interrogation of the very small detection region, which despite the small size of the chip, makes them generally unsuitable for portable analytical applications. However, Ogilvie et al. [40] have shown proof of concept for a multiplexed stop-flow microfluidic system for the colorimetric detection of phosphate in which simple light-emitting diode (LED) detectors were used. The chip was prepared by micromachining poly(methylmethacrylate) plates that were bonded and polished using solvent vapor, giving flow channels of 160×300 μm and 300×300 μm for the photometric detection cell. While the current system appears to lack the sensitivity necessary for the determination of the low nutrient concentrations in marine waters, it comprises robust fluidic and detection components and shows great potential for autonomous, portable monitoring applications.

A recent development in the area of microfluidics has been the manufacture of paper-based devices [41], in which hydrophilic flow channels or zones are defined on a paper substrate by the application of hydrophobic materials (e.g., waxes, sizing agents, etc.). When an aqueous sample is spotted onto the device, it is transported to a reagent-treated zone by capillary action, and thence to a detection zone, where visual, photometric [42], electrochemical [43], or other modes of detection are used for quantification. The geometry of the detection region in paper-based devices is typically of the order of several square millimeters, making detection by common imaging techniques or even by the naked eye, quite feasible.

Many of the paper-based methods described to date have been designed for clinical determinations, e.g., glucose [42–45], protein [42,44], and ketones [45] in blood or urine, but more recently, the potential value of this approach as a low-cost environmental monitoring tool has been recognized.

Jayawardane et al. [46] have reported the development of a paper-based method for the determination of reactive phosphate in waters and wastewaters. Unlike other methods where reagent and sample mixing is achieved by lateral flow, constrained within hydrophilic channels, this approach uses transverse flow though adjacent layers of paper, each of which has a hydrophilic zone defined by inkjet printing of a hydrophobic sizing agent (Fig. 6). For the determination of phosphate, μL amounts of mixed acidic ammonium molybdate and potassium antimonyl tartrate, and ascorbic acid are added to zones 1 and 2 (Fig. 6), respectively. After controlled drying, the paper is folded so that zones 1 and 2 are adjacent, and then laminated to produce a credit card-sized sheet capable of performing 15 individual determinations of phosphate. Sample addition is made by pipetting 10 μL of sample into small holes

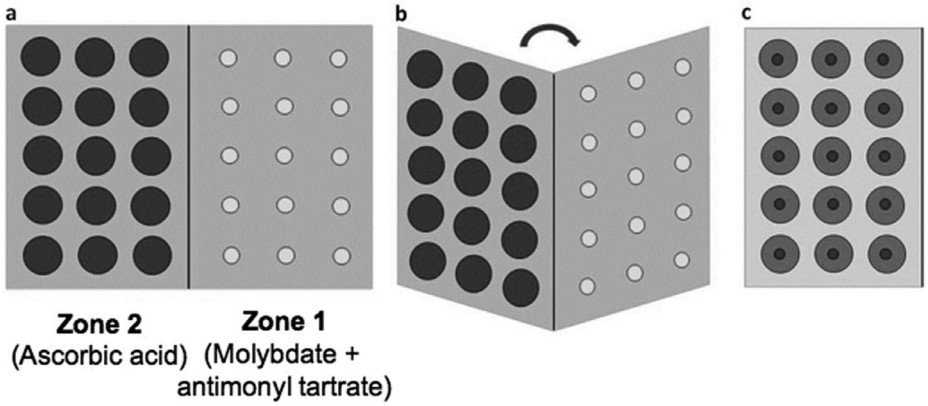

Fig. 6 Diagram showing the sequence of operations in the preparation of a paper-based device for determination of phosphate: (a) Inkjet-printed paper with reagents applied to zones 1 and 2, (b) paper folded so that centers of zones 1 and 2 align, (c) folded paper laminated and holes punched in laminate to allow sample addition to zone 2.

in the laminate made using a biopsy punch. Measurable development of phospholybdenum blue occurs within minutes, and quantification is achieved using a flatbed scanner on-site, in conjunction with an image analysis program, such as ImageJ [47] (Fig. 7). This approach provides the facility to perform rapid on-site determination of reactive phosphate with a linear response up to 10 mg L^{-1} P and a detection limit of 0.2 mg L^{-1} P. The repeatability at 5 mg L^{-1} P is <2 %, and 15 replicate determinations can be made 5 min after sample addition. The paper-based devices are inexpensive to use (<3 cents per determination), and have a usable life of >90 days if stored frozen. From a green chemical perspective, each determination requires a fraction of the reagents ($1/200^{th}$ to $1/4000^{th}$) compared with the conventional batch chemical methods. They are technically simple to use, and when used in conjunction with a flatbed scanner, provide more sensitive and reliable measurements of phosphate than those attainable using commercial test strips. The development and further application of these paper-based devices is an example of how modern information technologies can be combined synergistically with fundamental chemistry to provide high-quality, accessible analytical data with a minimum of cost and operator expertise.

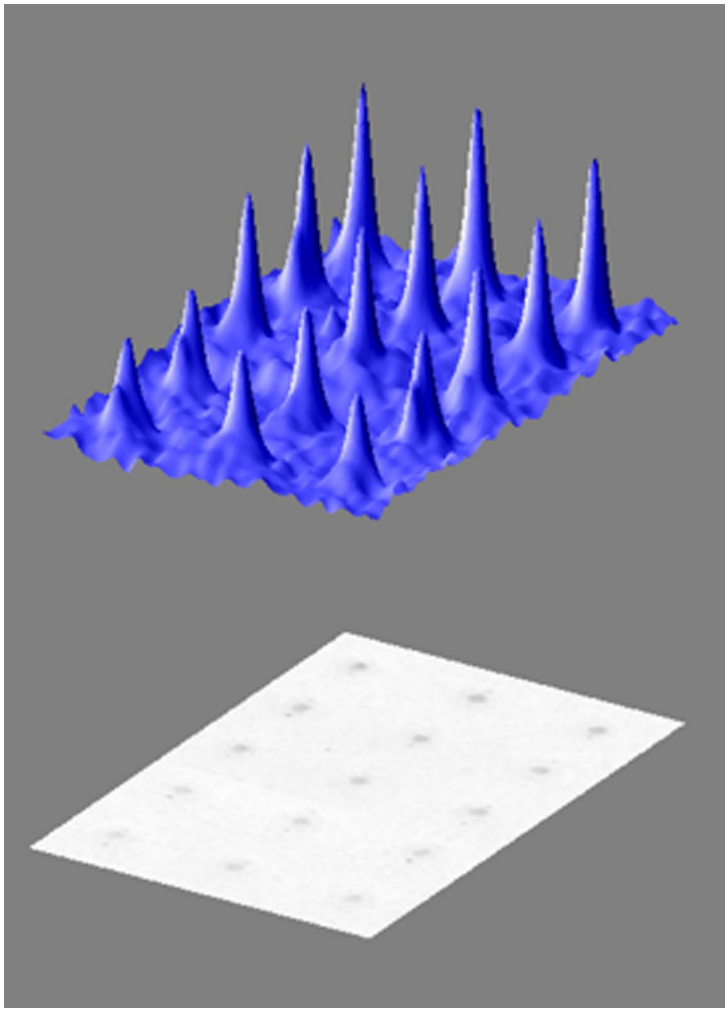

Fig. 7 Scanned image of a paper-based device after addition of 0.1–1.0 mgP L^{-1} phosphate standards with corresponding 3D densimetric image from which calibration data are obtained.

CONCLUSIONS

This paper selectively reviews areas of current analytical chemistry research that have the potential to give *more with less* for nutrient monitoring in aquatic systems, i.e., improved intensity, frequency, or quality of monitoring data with less cost, complexity, or operator involvement. While flow analysis techniques are well established in nutrient analysis laboratories, there is a strong case for applying this technology in the field, using instruments designed for the purpose, in order to obtain better information on the distribution and fluxes of nutrient species. Paper-based and microfluidic techniques, in general, are still emerging fields of research. However, there is great potential for the application of simple, truly compact, reagent-efficient devices that can be applied to nutrient monitoring for minimal cost, especially in areas that involve water quality monitoring by community groups or agricultural communities, or for enhanced monitoring of licensed discharges by industry and environmental regulators.

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